Methods: Chemically synthetic siRNA targeting CTGF was transfected into HSC T6 and injected into rats' liver by intraportal vein, and meantime these rats also received carbon tetrachloride (CCl_4) by subcutaneous injections every three days for 6 consecutive weeks, untreated HSC T6 or/and rats with random siRNA treatment as control. Total RNA or/and protein in HSC T6 and rats hepatic tissue were extracted respectively. The expression of CTGF and TGF-β_1, Smad2, 3 and 7 mRNA were detected by means of reverse transcription-polymerase chain reaction (RT-PCR) and/or Western blot respectively.
Results: CTGF siRNA significantly reduced the expression of CTGF protein in HSC T6. At 48 h after CTGF siRNA treatment, the down-regulation of CTGF protein was the most significant, up to 94±4% ($t = 46.1961$, $p < 0.01$), but the expressions of TGF-β_1, Smad2, 3 and 7 mRNA have no different in HSC T6 compared with blank control. Six weeks after CCl_4 injection, prominent upregulations were observed in the gene expressions of CTGF and transforming growth factor-β_1 (TGF-β_1) in saline or control siRNA-treated rats livers. Administrating CTGF siRNA for six weeks, by contrast, markedly attenuated the induction of CTGF and TGF-β_1 genes, the expression of CTGF and TGF-β_1 protein decreased by 95±2% ($F = 21.234$, $p < 0.01$) and 74±8% ($F < 13.4643$, $p < 0.05$), respectively, whereas Smad2, 7 proteins expression were not affected.
Conclusion: Silence of CTGF gene could have repreesion role of TGF-β/Smads signal in rats liver.

OL-020 Sustained and high expression of hHGF in mice following repeated hydrodynamic injections of naked plasmid

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Objective: To construct human HGF expression vector (pCMV-hHGF) and characterize sustained expression of pMD-hHGF in vivo by repeated hydrodynamic injections.
Methods: Total RNA was extracted from human liver, cDNA was obtained by reverse transcription, hHGF cDNA was amplified and cloned into pMD18-T vector and the sequence were ensured by retriction endonucleases and sequencing assay. hHGF gene was dissected from pMD-hHGF and recloned into pcDNA3.0. pCMV-hHGF was analyzed by restriction endonucleases to ensure the orientation. After the plasmid was transfected into mouse livers by repeated hydrodynamic injections, we collected plasma and livers of mouse at the different time point, and then detected the expression of hHGF by ELISA.

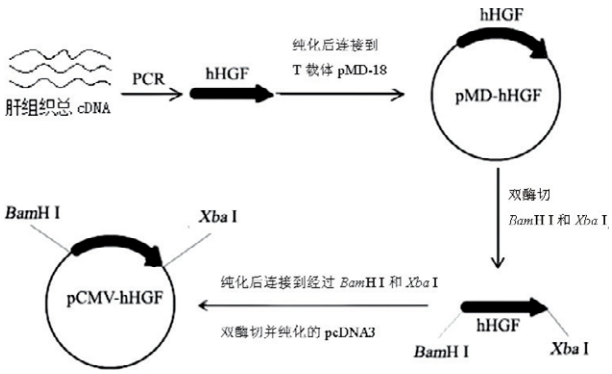

Fig. 1. Construction process of recombinant vector pCMV-hHGF

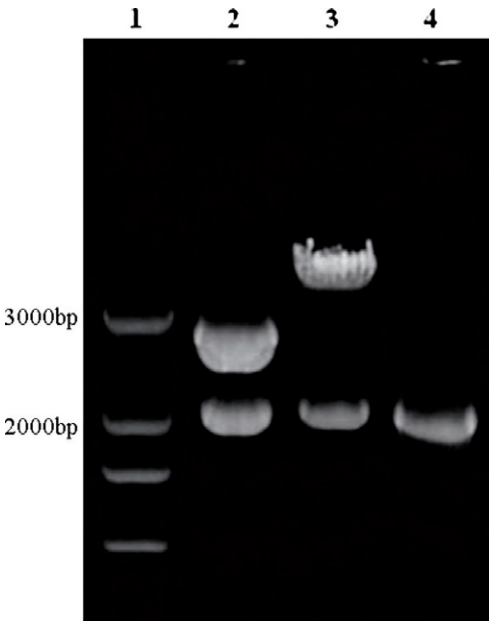

Fig. 2. Enzymatic digestion analysis of recombinated plasmid. 1: DNA maker; 2: pCMV-hHGF + XbaI + BamHI; 3: pMD-hHGF + XbaI + BamHI; 4: PCR amplification product (2187bp).

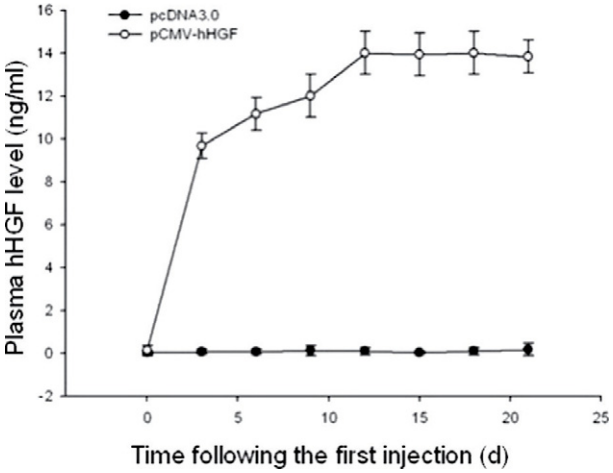

Fig. 3. The level of Plasma hHGF detected by ELISA.

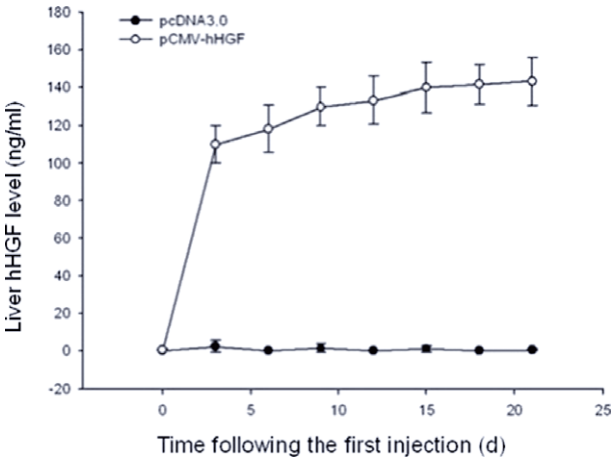

Fig. 4. The level of liver hHGF detected by ELISA.

Results:A 2187bp gene fragment was obtained and coloned into pMD18-T vector, and the sequence was correct. hHGF gene was subcloned into pcDNA3.0 vector, and then restriction endonucleases assays showed the correct orientation. At the different time point post the first hydrodynamic injection, the expression of hHGF could be detected by ELISA.
Conclusion: hHGF expression vector (pCMV-hHGF) has been successfully constructed and repeated hydrodynamic injections can promote sustained and high expression of hHGF in vivo.
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OL-021 The difference in serum TNF-alpha and hepatocyte apoptosis between rats with acute liver failure and acute-on-chronic liver failure

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Background: Tumor necrosis factor (TNF) α plays a pivotal role in pathogenesis of D-Galactosamine (D-Gal) and lipopolysaccharide (LPS)-induced liver injury.The present study was designed to investigate changes of serum TNF-α and hepatocyte apoptosis in D-Gal/LPS induced acute-on-chronic liver failure (ACLF) and acute liver failure (ALF).
Method: Sixty-five rats with immunologic hepatic fibrosis acted as ACLF group, and 50 healthy female Wistar rats as AHF group. D-Gal (400 mg/kg) and LPS (100 μg/kg) were intraperitoneally administered in the two groups. Ten rats in ACLF group and 6 rats in AHF group were sacrificed at 0, 4, 8, and 12 hours, respectively. Blood were taken for liver function tests and TNF-α levels. Cell apoptosis was detected by tunnel assay. Mortality and survival time were recorded in the two groups.
Results: Mortality rates were 88.0% and 58.3%, respectively, in rats with ACLF and acute liver failure ($P = 0.028$), with mean survival time of 15.6 $\pm$ 1.8 hours, and 16.1 $\pm$ 3.7 hours, respectively. Histology of liver section revealed massive or submassive necrosis in regenerative nodules, while fibrosis septa were intact in ACLF rats. Compared with those in group of acute liver failure, plasma TNF-α levels were significantly lower and arrived its peak later. Index of apoptosis was significantly higher in ACLF group at each time points than group of acute liver failure.
Conclusion: Animal model of acute-on-chronic liver failure can be established by treatment human serum albumin-induced rat's cirrhosis with D-gal/LPS. When cirrhosis and healthy rats suffered from acute liver attack, there were difference in inflammatory reaction, extent of cell apoptosis and changes of subcellular structure.

OL-022 Alpha fetoprotein functions as an inhibitor on the transduction of caspase-3 signaling pathway in human hepatoma Bel 7402 cells

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Objective: Many evidences indicate that infected with hepatitis virus B or C concomitants the high expression of alpha-fetoprotein (AFP) in hepatic cells, AFP is a tumor marker of hepatoma, documents indicated that AFP has a property to maintain the growth of hepetocellular cancinoma cells (HCC) in vivo, but the critical functional step of AFP still obscurity, the present investigation in order to explore the influence AFP on the transduction of apoptotic signal in HCC and the role mechanism of AFP counteracts the apoptosis induced of HCC by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL).
Methods: Laser confocal microscopy was used to observe co-localization of AFP and caspase-3 or AFP and caspase-8; Co-immunoprecipitation (Co-IP) was utilized to analyze AFP interacted with intracellular apoptotic signal molecules caspase-3 or caspase-8; Short small RNA interfering (siRNA) was applied to knockdown the expression of AFP in Bel 7402 cells; AFP influences the activity of caspase-3 or caspase-8 was detected by protease activity colorimetric methods; MTT was utilized to analyze the growth and fluorescent microscopy was used to observe the apoptosis of Bel 7402 cells after knockdown the expression of AFP followed treatment with TRAIL (2 nmol/L) for 24 h.
Results: It showed that AFP has a property to interact with caspase-3 in cytoplasm, but could not binding with caspase-8; The activity of caspase-3 or caspase-8 was enhanced when treated with ATRA (40μmol/L) plus TRAIL (2nmol/L) for 24 h, but treated with TRAIL (2 nmol/L) alone for 24 h, the activity of caspase-8 was promoted but the activity of caspase-3 has not any alteration; AFP was able to inhibit the activity of caspase-3 but it has any affect on the activity of caspase-8 in vitro; When knockdown the expression of AFP by RNAi followed treatment with TRAIL 2nmol/L) for 24 h, the activity of caspase-3 was increased but the activity of caspase-8 had not any change, MTT detecting showed that the growth of Bel 7402 cells was inhibited (ratio 48.4%), and TRAIL (2 nmol/L) could induce the apoptosis of the cancer cells.
Conclusions: AFP has a capacity to block the transduction of apoptotic signal attribute to it has a property to inhibit the activity of caspase-3, this is also the pivotal events that AFP counteracts the apoptosis-induced by TRAIL of hepatoma cells.

OL-023 Effect of *Bifidabacterium* and *Lactobacillus* on endotoxemia in rats with ischemia/reperfusion liver injury

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Background and Aims: This study was performed to investigate whether supplement of *Bifidabacterium* and *Lactobacillus* could improvement endotoxemia and liver function in rats with I/R liver injury and the mechanisms involved.
Methods: Thirty-seven adult Sprague-Dawley in SPF grade rats (200$\pm$10g) were randomized into four groups: including Group 1 (sham group, n = 6), Group 2 (ischemia/reperfusion group, n = 10), Group 3 (BIF group, n = 10) and Group 4 (LACT group, n = 11). Rats received *Bifidobacterium ZYB0401* (1.2×10^9 cfu/day) or *Lactobacillus ZYL0401* (1.2×10^9 cfu/day) respectively in Group 3, Group 4 respectively, or physiological saline in Group 1 and Group 2 for a week. On the sixth day, rats except for in Control or Sham group were subjected to 20 min of liver ischemia, and rats in Sham group were only subjected to sham operation. Twenty-two hours later, rats were sacrificed and liver enzymes, plasma endotoxin, serum tumor necrosis factor-α (TNF-α), intestinal bacterial count, intestinal mucosal histology was studied.
Results: Plasma endotoxin increased significantly in rats subjected to Ischemia-reperfusion, associated with liver enzymes elevation ($p < 0.01$). Intestinal *Bifidobacterium* and *Lactobacillus* decreased ($p < 0.01$) and Intestinal *Enterobacteria* and *Enterococci* ($p < 0.05$) increased in Model